J. Clin. Chem. Clin. Biochem.
Vol. 24, 1986, pp. 19–33

Catalytic Enzyme Activity Concentration in Thoracic Duct, Liver, and Intestinal Lymph of the Dog, the Rabbit, the Rat and the Mouse

Approach to a Quantitative Diagnostic Enzymology, II. Communication

By *J. Lindena, W. Küpper** and *I. Trautschold†*

*Abteilung Klinische Biochemie und *Abteilung für Versuchstierkunde, Medizinische Hochschule Hannover*

(Received April 26/November 18, 1985)

In memoriam Professor Dr. Dr. Ivar Trautschold († 31. 01. 1984)

Summary: In the mixed body lymph of the thoracic duct and in the defined organ lymph of the liver and the intestine, the catalytic activity concentrations of up to sixteen enzymes and the concentrations of albumin and protein were determined, as well as the transport rate of these substances and their lymph/plasma ratio. Thoracic duct lymph specimens were obtained from an extracorporeal lymph shunt in anaesthetized and conscious dogs and from short-term fistulas in anaesthetized rabbits, rats and mice. Additionally, rabbits and rats underwent passive motion of the hind limbs in another experimental trial. Thoracic duct flow in anaesthetized dogs is only half that seen in conscious dogs, due to bypassed muscular lymph. A similar flow change is seen during passive motion of hind limbs in anaesthetized rabbits and rats. From a literature review of flow in the four main lymphatics of the body, it is concluded that the thoracic duct flow should account for 50–70% of total body lymph flow. In the anaesthetized state, flow is mainly of visceral origin. In the conscious state and during passive motion the increased flow is of muscular origin. In the latter case, the catalytic activities of enzymes like lactate dehydrogenase, malate dehydrogenase, creatine kinase, aldolase and phosphohexose isomerase, increase in lymph as does their lymph/plasma ratio. These enzymes have high catalytic activities in muscle. Their transport into the blood increases 2–3-fold, due to a doubling of lymph flow. Reported data for anaesthetized and immobile animals therefore far underestimate the significance of thoracic duct enzyme transport. Liver lymph was obtained from anaesthetized dogs and rabbits. Our finding that lymph catalytic activity for several enzymes is higher than in plasma is not compatible with the proposed delivery of plasma proteins directly into the sinusoidal space without prior mixing with the Space of *Disse*. Enzymes in liver lymph should derive from parenchymal and endothelial lining cells. Their site of delivery from the hepatocyte seems different from that of proteins. Liver lymph is an important transport route of enzymes into the blood. Intestinal lymph was sampled from anaesthetized dogs, rabbits and rats. It was shown that most enzymes from the intestine are primarily released into the interstitial space and from there are transported via the lymph into the blood.

Katalytische Enzymaktivitätskonzentration in Ductus Thoracicus-, Leber- und Intestinallymphe bei Hund, Kaninchen, Ratte and Maus
Versuch der Begründung einer quantitativen Diagnostischen Enzymologie, II.

Zusammenfassung: In der Mischlymphe des Ductus Thoracicus und in den definierten Organlymphen von Leber und Intestinum wurden die katalytischen Konzentrationen von bis zu 16 Enzymen und die Konzentra-

tionen von Albumin und Protein bestimmt, sowie deren Transport und ihr Lymph/Plasma-Quotient. Ductus Thoracicus-Proben wurden über einen extrakorporalen Lymphshunt von anaesthesierten und wachen Hunden gewonnen und von kurzzeit gefistelten anaesthesierten Kaninchen, Ratten und Mäusen. Bei Kaninchen und Ratten wurden in einem weiteren experimentellen Ansatz die Hinterextremitäten passiv bewegt. Der Ductus Thoracicus-Lymphfluß macht bei anaesthesierten Hunden nur die Hälfte des Flusses von wachen Tieren aus, weil die Muskellymphe ausgespart bleibt. Eine ähnliche Änderung des Flusses ist zu beobachten, wenn bei anaesthesierten Kaninchen und Ratten die Hintergliedmaßen passiv bewegt werden. Aus einer Literaturübersicht der Lymphflüsse der vier Hauptlymphsammelgefäße des Körpers wird gefolgert, daß der Ductus Thoracicus-Lymphfluß 50–70% der Gesamtkörperlymphe ausmacht. Unter Anaesthesie ist der Fluß im wesentlichen visceralen Ursprungs. Im wachen Zustand und bei passiver Bewegung ist der gesteigerte Fluß dem Muskel zuzuschreiben. Hierbei steigen die katalytischen Aktivitäten von Enzymen, die mit hoher katalytischer Aktivität im Skelettmuskel vorkommen, wie Lactatdehydrogenase, Malatdehydrogenase, Kreatinkinase, Aldolase und Phosphohexoseisomerase, in der Lymphe an; ebenso steigt ihr Lymph/Plasma-Quotient; ihr Transport in das Blut steigt aufgrund des verdoppelten Lymphflusses auf das 2–3-fache an. Die bisher nur von anaesthesierten bzw. immobilisierten Tieren mitgeteilten Ergebnisse unterschätzen daher bei weitem die Bedeutung des lymphatischen Transports von Enzymen über den Ductus Thoracicus. Leberlymphe wurde von anaesthesierten Hunden und Kaninchen gewonnen. Wir fanden für zahlreiche Enzyme höhere katalytische Aktivitäten in der Lymphe als im Plasma, was mit dem in der Literatur vorgeschlagenen Mechanismus der Freisetzung von Plasmaproteinen direkt in den Sinusoidalraum ohne vorherige Mischung mit dem *Disse*'schen Raum nicht vereinbar ist. Enzyme in der Leberlymphe sollten aus Parenchymzellen und Endothelzellen stammen. Ihr Freisetzungsort aus dem Hepatocyten scheint sich von dem der Plasmaproteine zu unterscheiden. Leberlymphe stellt einen wichtigen Transportweg für Enzyme in das Blut dar. Intestinallymphe wurde von anaesthesierten Hunden, Kaninchen und Ratten gewonnen. Es wird gezeigt, daß die meisten Enzyme des Intestinums zuerst in das Interstitium gelangen und dann auf dem Lymphwege in das Blut transportiert werden.

Introduction

Enzymes continuously released from cells are thought to reach the intravascular space in three different ways: directly, if the cells of origin are in contact with the circulating blood (e. g. blood cells, endothelial cells); directly and indirectly if the cells have contact with the intravascular and interstitial spaces and/or if the capillary permeability is high (e. g. liver, spleen); indirectly if the cells are in contact with the interstitial space only and/or the capillary permeability is low (e. g. skeletal muscle, other parenchymal tissues) (1).

Interstitial fluid, reflected in the lymph, therefore contains enzymes from nearly the whole body. This lymph enters the blood via four great lymph collecting vessels. With respect to flow rate, the thoracic duct is the most important feeder. Thoracic duct lymph has been studied frequently in animals but also some data from man are available. In the latter case, however, lymph from pathological states only has been studied (surveys: l. c. (2, 3)). Whereas data on flow and protein composition are numerous, only meagre data are given about enzymatic composition. Besides the general and still ongoing interest in protein composition of different lymphatics, a special interest in lymph in the field of clinical enzymology has developed over the years. Lymph has been accepted as an important transport route of cellular enzymes from tissues to the intravascular space (survey: l. c. (2)). A relatively small number of enzymes have been investigated, however, and these have often not included the diagnostically important enzymes. Additionally, all the studies reported so far have been performed in anaesthetized animals whose thoracic duct lymph flow was therefore significantly reduced (4, 5). Long-term thoracic duct fistulas on the other hand lead to a continuous loss of large amounts of fluid and protein. Therefore, quantitative conclusions cannot be drawn from these studies.

In dogs we report on the thoracic duct flow and the enzymatic composition of extracorporeal lymph from conscious vs. anaesthetized animals with lymph shunts. In anaesthetized rabbits and rats, the hind limbs were moved passively as an additional experimental trial. It was anticipated that this would stimulate the flow and the composition in a manner similar to that seen in conscious dogs (6, 7). In anaesthetized mice, a short-term fistula technique of the thoracic duct was used.

Data on thoracic duct transport of diagnostically important enzymes in conscious dogs, in rabbits, in rats and in mice have not been reported.

Since the lymph of the thoracic duct is a mixture of interstitial fluid from various sources in the body, no direct conclusions can be drawn as to the origin of enzymes in it. Intestinal and hepatic lymph, however, drain a well defined region and information about their physiologic catalytic activities should contribute to an understanding of the organs of origin for these enzymes. For the liver, the most important organ in diagnostic enzymology, quantitative data on lymph enzymes is lacking.

All studies reported were performed with the four most frequently used animals in experimental medicine to provide a more reliable basis for possible comparison with and application to man. Thoracic duct, hepatic and intestinal lymph from healthy man is not accessible for ethical reasons.

Materials and Methods

Dogs, rabbits, rats and mice were used. Strain, housing conditions and diet have been described in a foregoing publication (8). Varying lymph collecting vessels were cannulated in the animals.

Dogs

For insertion of the cannula dogs were anaesthetized by intravenous injection of sodium pentobarbital (Narcoren®, 30 mg/kg body weight) after premedication with phenothiazine (Combelen®) and methadone hydrochloride (Polamivet®). Anaesthesia was maintained with halothane.

Thoracic duct

A modified T-tube of Silastic (Dow Corning, USA; i. d. 0.76 mm, o. d. 1.65 mm) was inserted as a thoracic duct-to-duct shunt in the neck according to *Girardet & Benninghoff* (9). Briefly, the principle behind this method consists of exteriorizing the entire thoracic duct circulation by placing the ends of the short arm of a T-tube into the afferent and efferent thoracic duct. The long arm of the T-tube is then used for sampling the flow through this shunt. An intravenous placement unit (BARDI-CATH, BARD, GB) was inserted into the right external jugular vein for blood sampling. We report on two experimental groups.

Anaesthetized dogs (24 observations in 12 dogs, 25—42 kg body weight, $\bar{x}$ = 32 kg): After successful insertion of the lymph shunt, lymph was immediately quantitatively collected for two consecutive 30 min intervals. Blood was withdrawn at the end of each sampling period.

Conscious dogs (27 observations in 7 dogs, 20—40 kg body weight, $\bar{x}$ = 31 kg): Seven days after surgery, when catalytic activities in plasma had returned to normal, lymph sampling for analytical purposes was performed twice daily at approximately the same times for each dog in the morning and afternoon. The dogs were awake, unrestrained and standing. Lymph was collected for a 5 min period. A corresponding jugular vein blood sample was taken. Between lymph sampling in the conscious dogs, the long arm of the T-Tube was clamped.

Liver lymph

The lymphatics emerge from the hilus of the liver and, anastomosing freely over the surface of the gastrohepatic ligament, run caudally to the hepatic lymph node adjacent to the portal vein (10). The largest efferent lymphatic was cannulated (Silastic, i. d. 0.76 mm, o. d. 1.65 mm). The others were ligated or coagulated. Lymph was immediately collected for two consecutive 15 min intervals. Corresponding jugular vein blood samples were taken. We report on 26 observations in 13 dogs (23—34 kg body weight, $\bar{x}$ = 27 kg).

Intestinal lymph

There are several (usually two) trunks. They course from the intestine with the cranial mesentery artery and anastomose with each other freely (11). We cannulated one or both trunks with Silastic catheters (i. d. 0.76 mm, o. d. 1.65 mm). Any other trunks were occluded. Lymph was immediately sampled for two consecutive 15 min intervals with corresponding blood samples.

Lymph from liver and intestine were sampled, if possible, simultaneously. We report on 27 observations in 14 dogs (24.5—40 kg body weight, $\bar{x}$ = 31 kg).

Rabbits

Animals were anaesthetized by intravenous injection of sodium pentobarbital (Narcoren®, 20 mg/kg body weight) after premedication with ketamine hydrochloride (Ketavet®, 15 mg/kg body weight). Anaesthesia was maintained with halothane.

Thoracic duct

The duct was cannulated with a Silastic tube (i. d. 0.57 mm, o. d. 0.94 mm) at its entry into the jugulo-subclavian junction, leaving the pleural cavity unviolated (modified from l. c. (12)). We report on three experimental groups.

Anaesthetized rabbits (40 observations in 20 rabbits, 4.0—4.5 kg body weight, $\bar{x}$ = 4.3 kg): The spontaneously flowing lymph was collected for two consecutive 15 min periods.

Anaesthetized rabbits with passive motion of hind limbs (10 rabbits, 4.0—4.3 kg body weight, $\bar{x}$ = 4.2 kg): Both hind limbs were moved passively by flexing and extending them manually with a frequency of 60/min. Lymph was sampled for about 20 min.

Anaesthetized rabbits after passive motion of hind limbs: after the end of passive motion, the spontaneously flowing lymph was sampled for another 20 min.

Liver lymph

Hepatic lymph was collected from an efferent vessel coursing over the pancreas after emerging from the main hepatic lymph node which lies adjacent to the portal vein near the porta hepatis (13). Smaller lymph vessels were ligated. Lymph was collected with a Silastic tube (i. d. 0.57 mm, o. d. 0.94 mm) for two consecutive 15 min periods with corresponding arterial blood samples. We report 12 observations in 6 animals (4.0—4.9 kg body weight, $\bar{x}$ = 4.4 kg).

Intestinal lymph

There is no description of rabbit intestinal lymph collection in the literature. The intestinal lymph vessel anatomy is similar to that described for dogs and rats. We sampled lymph for two consecutive 15 min periods with a Silastic tube (i. d. 0.57 mm, o. d. 0.97 mm) with corresponding arterial blood samples. We report 16 observations in 8 rabbits (4.0—4.6 kg body weight, $\bar{x}$ = 4.3 kg).

Rats

Rats were anaesthetized and a polyethylene catheter for blood sampling was inserted into the carotid artery as described (8).

Thoracic duct

The cervical approach at the root of the neck as outlined by *Saldeen & Linder* (14), was used. Lymph cannulation was carried out under a *Zeiss* IV/b stereo-microscope (Carl Zeiss, Oberkochen) with a zoom attachment, using a four point incident illuminator fixed under the objective, with fibre-conducted cold light from a step down transformer (KL 150 B; Schott, Mainz). During lymph collection the animals were placed on a heated table at 37 °C and lymph was sampled with a Silastic tube (i. d. 0.57 mm, o. d. 0.94 mm) or through a polyethylene catheter (Portex, Great Britain, i. d. 0.58 mm, o. d. 0.98 mm). Blood was drawn at the end of the lymph sampling period. We report on three experimental groups.

Anaesthetized rats (30 rats, 230–290 g body weight, $\bar{x}$ = 250 g): The spontaneously flowing lymph was sampled for between 40 and 70 min.

Anaesthetized rats with passive motion of hind limbs (14 rats, 250–280 g body weight, $\bar{x}$ = 260 g): Both hind limbs were moved passively by manual flexion and extension at a frequency of 60 min^{-1}. Lymph was sampled for about 15 min.

Anaesthetized rats after passive motion of hind limbs (11 rats, 250–280 g body weight, $\bar{x}$ = 270 g): After the end of passive motion, the spontaneously flowing lymph was sampled for another 20 min.

Intestinal lymph

We followed detailed descriptions for intestinal lymph sampling in rats, given by *Warshaw* (15) and *Lee* (16). The Silastic tube (i. d. 0.57 mm, o. d. 0.94 mm) was inserted under the stereo microscope. Lymph was sampled for 40 to 60 min from animals lying on a heated table (37 °C). We report on lymph collection in 20 rats (215–270 g body weight, $\bar{x}$ = 240 g).

Mice

Mice were anaesthetized and a Silastic catheter for blood drawing was inserted into the jugular vein as described (8).

Thoracic duct

A cervical approach to thoracic duct lymph in mice was recently outlined by us (17). A Silastic catheter (i. d. 0.30 mm, o. d. 0.64 mm) was inserted into the duct proximal to the jugulo-subclavian junction under a stereo-microscope. Lymph was collected for between 30 to 40 min from mice placed on a heated table (37 °C). At the end of the lymph sampling, blood was drawn. We report of lymph collection in 14 mice (28–31 g body weight, $\bar{x}$ = 30 g).

General technical remarks and remarks about lymph and blood preparation

Preliminary experiments were done to ensure that the operative procedure for cannula insertion did not alter catalytic activity in plasma during the course of the sampling period in the acute experiments. The operative procedure produced no changes.

The inserted lymph cannulae were fixed in place by small droplets of the tissue adhesive isobutyl 2-cyanoacrylate (Ethicon, Norderstedt). Lymph was collected in preweighed test tubes containing minute amounts of heparin. Lymph flow was determined gravimetrically. Lymph then was centrifuged at 12 000 *g* for 2 min. Lipaemic intestinal or thoracic duct lymph was clarified by using an Airfuge Ultracentrifuge with the rotor ACR-9 (Beckman Instruments) by flotation of the chylomicrons after 10 min of centrifugation at 107 000 *g*. Lymph plasma was stored at −70 °C.

Blood was sampled, and plasma prepared as described (8).

Determination of catalytic enzyme activity concentration, protein concentration and albumin concentration

The catalytic activities of the following enzymes were determined in lymph and plasma:

lactate dehydrogenase (EC 1.1.1.27)
malate dehydrogenase (EC 1.1.1.37)
isocitrate dehydrogenase (EC 1.1.1.42)
glutamate dehydrogenase (EC 1.4.1.3)
γ-glutamyl transferase (EC 2.3.2.2)
aspartate aminotransferase (EC 2.6.1.1)
alanine aminotransferase (EC 2.6.1.2)
creatine kinase (EC 2.7.3.2)
adenylate kinase (EC 2.7.4.3)
cholinesterase (EC 3.1.1.8)
alkaline phosphatase (3.1.3.1)
acid phophatase (EC 3.1.3.2)
α-amylase (EC 3.2.1.1)
amino acid arylamidase (EC 3.4.11.2)
aldolase (EC 4.1.2.13)
phosphohexose isomerase (EC 5.3.1.9)

Analytical methods for catalytic enzyme activity, protein and albumin concentrations and concurrent quality control have been described in the foregoing communication (8).

Results

Lymph flow

Data on lymph flow in the three lymph collecting vessels of dogs, rabbits, rats and mice under various experimental trials are presented in table 1.

In dogs, thoracic duct flow in conscious animals is more than twice that seen in anaesthetized animals. In anaesthetized dogs the flow of intestinal lymph exceeded thoracic duct lymph flow. This unusual finding is probably the result of intermittent abdominal massage used in intestinally cannulated animals to help maintain continuous lymph flow and to avoid crushing and compression of the catheters during the experiment.

Thoracic duct flow in anaesthetized rats and rabbits doubles during passive motion of the hind limbs. After passive motion, flow values are quite similar to those of immobile animals.

Tab. 1. Thoracic duct, liver, and intestinal lymph flow (ml/h; $\bar{x} \pm$ SEM) in the dog, the rabbit, the rat and the mouse during different experimental trials.

	Dog		Rabbit anaesthetized			Rat anaesthetized			Mouse anaesthetized
	anaesthetized	conscious	immobile	during motion	after motion	immobile	during motion	after motion	immobile
Thoracic duct	48.0 ± 6.0	112 ± 12.1	12.4 ± 1.2	23.4 ± 1.9	13.9 ± 1.1	1.06 ± 0.05	2.67 ± 0.15	1.24 ± 0.09	0.41 ± 0.01
Liver lymph	16.1 ± 1.8	–	1.1 ± 0.1	–	–	–	–	–	–
Intestinal lymph	74.6 ± 4.3	–	6.3 ± 0.5	–	–	0.70 ± 0.04	–	–	–

Thoracic duct: catalytic enzyme activity, lymph/plasma ratio, and enzyme transport

These results are summarized in table 2a, 2b and 2c, respectively. Our data on catalytic activity in lymph can be analysed only in relation to the corresponding catalytic activities in plasma for a given species. Different experiments in a distinct species can also be compared.

In anaesthetized dogs, lymph/plasma ratios below one are noted for cholinesterase, alkaline phosphatase, leucine arylamidase and protein. These ratios remain below one in the conscious state. In addition the alanine aminotransferase ratio becomes less than one. All other ratios, however, increase in conscious dogs.

In anaesthetized immobile rabbits, ratios of less than one are observed for glutamate dehydrogenase, γ-glutamyl transferase, cholinesterase, acid phosphatase, amino acid arylamidase as well as for protein and albumin. These ratios further decrease during passive motion. The ratio for isocitrate dehydrogenase becomes less than one. All other ratios, however, increase during passive motion of the hind limbs. After passive motion, values are similar to those of the immobile group.

In anaesthetized immobile rats, ratios of less than one are seen for aspartate aminotransferase, creatine kinase, aldolase and for protein and albumin. During and after passive motion lactate dehydrogenase, alanine aminotransferase, cholinesterase and alkaline phosphatase decrease below a ratio of one, whereas creatine kinase during passive motion markedly increase to a ratio of greater than one.

In anaesthetized mice ratios of less than one are seen for alanine aminotransferase, cholinesterase, alkaline phosphatase, α-amylase, amino acid arylamidase and for protein and albumin.

Lymphatic transport, or the combination of lymph flow and catalytic activity, exhibits marked differences for each of the different experimental animals.

In conscious dogs, lymphatic transport of enzymes (except alanine aminotransferase) and of protein takes place at a greater rate than in anaesthetized dogs (i. e. threefold higher for lactate dehydrogenase, aspartate aminotransferase, creatine kinase and aldolase).

During passive motion in rabbits, lymphatic transport of all enzymes as well as protein transport increases. A two- and threefold increase is seen for lactate dehydrogenase, malate dehydrogenase, aspartate aminotransferase, creatine kinase, adenylate kinase, α-amylase, aldolase and phosphohexose isomerase.

Tab. 2a. Catalytic enzyme activity concentrations (U/l; $\bar{x} \pm$ SEM), protein and albumin concentrations (g/l; $\bar{x} \pm$ SEM) in thoracic duct lymph of the dog, the rabbit, the rat and the mouse.

	Dog		Rabbit anaesthetized			Rat anaesthetized			Mouse anaesthetized
	anaesthetized	conscious	immobile	during motion	after motion	immobile	during motion	after motion	immobile
Lactate dehydrogenase	51.2 ± 6.6	63.3 ± 5.5	141 ± 17	145 ± 14	129 ± 14	43.4 ± 3.4	40.5 ± 5.4	37.6 ± 4.8	230 ± 33
Malate dehydrogenase	128 ± 12	147 ± 18	323 ± 27	351 ± 20	311 ± 22	41.1 ± 2.8	50.1 ± 6.0	40.0 ± 4.8	278 ± 34
Isocitrate dehydrogenase	–	–	39.4 ± 3.6	32.4 ± 2.3	36.3 ± 1.9	–	–	–	–
Glutamate dehydrogenase	–	–	1.5 ± 0.28	1.1 ± 0.19	1.3 ± 0.20	–	–	–	–
γ-Glutamyl transferase	–	–	2.5 ± 0.18	1.8 ± 0.13	2.3 ± 0.16	–	–	–	–
Aspartate aminotransferase	23.2 ± 1.6	40.5 ± 5.2	43.3 ± 5.2	48.2 ± 3.3	43.0 ± 4.1	27.4 ± 1.1	22.7 ± 1.3	23.3 ± 1.8	34.4 ± 3.3
Alanine aminotransferase	33.5 ± 3.2	14.1 ± 1.9	38.7 ± 3.4	26.2 ± 3.0	35.1 ± 2.6	21.7 ± 1.2	21.6 ± 1.1	20.1 ± 1.3	16.9 ± 0.73
Creatine kinase	124 ± 19	157 ± 25	300 ± 39	382 ± 35	315 ± 31	58.0 ± 4.9	91.9 ± 16	70.8 ± 8.8	121 ± 22
Adenylate kinase	–	–	93.7 ± 1	139 ± 10	105 ± 11	43.6 ± 4.4	24.7 ± 4.0	20.4 ± 2.4	–
Cholinesterase	1338 ± 105	950 ± 56	70.2 ± 2.6	51.3 ± 1.9	58.3 ± 2.3	82.2 ± 4.9	60.8 ± 5.8	62.5 ± 6.2	540 ± 32
Alkaline phosphatase	52.0 ± 3.6	58.4 ± 5.4	121 ± 7.3	120 ± 5.6	126 ± 3.9	453 ± 26	464 ± 49	418 ± 50	89.4 ± 5.31
Acid phosphatase	–	–	17.6 ± 1.2	13.2 ± 1.1	16.4 ± 1.4	–	–	–	–
α-Amylase	–	–	395 ± 25	401 ± 19	380 ± 15	–	–	–	1775 ± 98
Amino acid arylamidase	6.7 ± 0.50	6.2 ± 1.1	27.0 ± 1.2	25.3 ± 0.81	25.8 ± 0.79	–	–	–	5.90 ± 0.39
Aldolase	19.9 ± 0.30	29.6 ± 3.3	23.9 ± 2.1	27.4 ± 2.0	25.0 ± 1.9	8.1 ± 0.45	8.1 ± 0.72	6.5 ± 0.47	31.7 ± 4.5
Phosphohexose isomerase	88.3 ± 10	104 ± 18	316 ± 30	380 ± 25	303 ± 29	47.7 ± 3.2	53.1 ± 6.5	45.3 ± 6.0	189 ± 24
Protein	49.4 ± 0.62	49.6 ± 1.9	51.3 ± 1.8	49.6 ± 1.5	49.0 ± 1.2	39.5 ± 1.1	36.9 ± 0.95	32.9 ± 1.4	27.0 ± 1.1
Albumin	–	–	25.9 ± 0.80	24.3 ± 0.62	23.2 ± 1.1	19.3 ± 0.8	18.1 ± 0.90	15.6 ± 1.1	11.1 ± 0.45

Tab. 2b. Thoracic duct lymph/plasma catalytic activity and mass concentration ratios ($\bar{x} \pm$ SEM).

	Dog		Rabbit anaesthetized			Rat anaesthetized			Mouse anaesthetized
	anaesthetized	conscious	immobile	during motion	after motion	immobile	during motion	after motion	immobile
Lactate dehydrogenase	1.5 ± 0.13	1.9 ± 0.26	3.9 ± 0.47	4.1 ± 0.51	3.7 ± 0.41	1.3 ± 0.13	0.99 ± 0.13	0.92 ± 0.12	1.8 ± 0.25
Malate dehydrogenase	2.7 ± 0.30	3.1 ± 0.49	1.1 ± 0.55	1.2 ± 0.41	1.0 ± 0.37	2.0 ± 0.17	2.0 ± 0.23	1.56 ± 0.12	2.7 ± 0.39
Isocitrate dehydrogenase	–	–	1.1 ± 0.10	0.87 ± 0.11	1.1 ± 0.09	–	–	–	–
Glutamate dehydrogenase	–	–	0.27 ± 0.01	0.19 ± 0.01	0.23 ± 0.01	–	–	–	–
γ-Glutamyl transferase	–	–	0.66 ± 0.04	0.43 ± 0.03	0.62 ± 0.04	–	–	–	–
Aspartate aminotransferase	2.0 ± 0.22	2.9 ± 0.31	2.6 ± 0.30	2.8 ± 0.25	2.5 ± 0.26	0.85 ± 0.04	0.76 ± 0.04	0.78 ± 0.06	1.9 ± 0.18
Alanine aminotransferase	1.4 ± 0.20	0.51 ± 0.06	1.1 ± 0.08	0.67 ± 0.03	0.94 ± 0.05	1.1 ± 0.07	0.92 ± 0.05	0.86 ± 0.06	0.87 ± 0.04
Creatine kinase	3.7 ± 0.34	5.7 ± 0.63	3.3 ± 0.42	4.1 ± 0.50	3.4 ± 0.20	0.76 ± 0.06	1.2 ± 0.20	0.98 ± 0.14	2.7 ± 0.30
Adenylate kinase	–	–	3.8 ± 0.38	5.9 ± 0.60	4.8 ± 0.22	1.83 ± 0.12	0.87 ± 0.14	0.71 ± 0.08	–
Cholinesterase	1.0 ± 0.19	0.69 ± 0.08	0.73 ± 0.02	0.54 ± 0.04	0.69 ± 0.03	1.1 ± 0.09	0.74 ± 0.07	0.77 ± 0.08	0.30 ± 0.02
Alkaline phosphatase	0.74 ± 0.10	0.78 ± 0.09	1.8 ± 0.12	1.4 ± 0.23	1.7 ± 0.29	1.0 ± 0.07	0.78 ± 0.08	0.70 ± 0.08	0.58 ± 0.03
Acid phosphatase	–	–	0.72 ± 0.03	0.55 ± 0.06	0.70 ± 0.09	–	–	–	–
α-Amylase	–	–	1.4 ± 0.07	1.2 ± 0.30	1.3 ± 0.21	–	–	–	0.83 ± 0.05
Amino acid arylamidase	0.91 ± 0.11	0.81 ± 0.10	0.76 ± 0.03	0.65 ± 0.40	0.78 ± 0.63	–	–	–	0.40 ± 0.01
Aldolase	1.8 ± 0.25	2.4 ± 0.38	1.6 ± 0.14	1.8 ± 0.30	1.5 ± 0.34	0.61 ± 0.04	0.52 ± 0.05	0.42 ± 0.03	2.5 ± 0.39
Phosphohexose isomerase	1.5 ± 0.22	2.2 ± 0.29	1.3 ± 0.13	1.7 ± 0.23	1.4 ± 0.19	1.1 ± 0.10	1.1 ± 0.13	0.90 ± 0.12	2.1 ± 0.26
Protein	0.70 ± 0.09	0.65 ± 0.06	0.80 ± 0.04	0.69 ± 0.02	0.75 ± 0.02	0.58 ± 0.02	0.52 ± 0.01	0.46 ± 0.02	0.45 ± 0.04
Albumin	–	–	0.75 ± 0.03	0.70 ± 0.01	0.71 ± 0.02	0.54 ± 0.02	0.52 ± 0.03	0.45 ± 0.03	0.36 ± 0.03

Tab. 2c. Lymphatic transport of enzymes (U/h; $\bar{x} \pm$ SEM), and of protein and albumin (g/h; $\bar{x} \pm$ SEM) in the thoracic duct.

	Dog		Rabbit anaesthetized			Rat anaesthetized			Mouse anaesthetized
	anaesthetized	conscious	immobile	during motion	after motion	immobile	during motion	after motion	immobile
Lactate dehydrogenase	2.46 ± 0.32	7.09 ± 0.62	1.66 ± 0.21	3.32 ± 0.30	1.72 ± 0.25	0.045 ± 0.003	0.101 ± 0.010	0.044 ± 0.004	0.082 ± 0.011
Malate dehydrogenase	6.72 ± 1.0	13.8 ± 1.8	3.79 ± 0.38	8.04 ± 0.59	4.32 ± 0.21	0.043 ± 0.003	0.127 ± 0.012	0.048 ± 0.006	0.107 ± 0.014
Isocitrate dehydrogenase	–	–	0.45 ± 0.04	0.77 ± 0.08	0.48 ± 0.03	–	–	–	–
Glutamate dehydrogenase	–	–	0.02 ± 0.001	0.03 ± 0.02	0.02 ± 0.001	–	–	–	–
γ-Glutamyl transferase	–	–	0.025 ± 0.002	0.04 ± 0.002	0.03 ± 0.002	–	–	–	–
Aspartate aminotransferase	1.03 ± 0.17	3.89 ± 0.53	0.54 ± 0.06	1.13 ± 0.11	0.56 ± 0.08	0.032 ± 0.005	0.059 ± 0.009	0.028 ± 0.002	0.013 ± 0.001
Alanine aminotransferase	1.54 ± 0.28	1.18 ± 0.09	0.51 ± 0.06	0.63 ± 0.06	0.46 ± 0.05	0.028 ± 0.005	0.059 ± 0.005	0.038 ± 0.012	0.007 ± 0.004
Creatine kinase	5.95 ± 0.91	17.6 ± 2.8	4.05 ± 0.48	8.84 ± 0.71	4.10 ± 0.31	0.068 ± 0.008	0.23 ± 0.03	0.084 ± 0.008	0.044 ± 0.005
Adenylate kinase	–	–	0.90 ± 0.09	2.93 ± 0.16	1.33 ± 0.07	0.043 ± 0.004	0.061 ± 0.007	0.024 ± 0.003	–
Cholinesterase	75.0 ± 7.6	116 ± 2.8	0.87 ± 0.07	1.14 ± 0.09	0.75 ± 0.05	0.085 ± 0.006	0.16 ± 0.015	0.076 ± 0.009	0.214 ± 0.016
Alkaline phosphatase	2.80 ± 0.49	5.25 ± 0.38	1.41 ± 0.09	2.60 ± 0.11	1.63 ± 0.10	0.47 ± 0.026	1.15 ± 0.12	0.503 ± 0.046	0.038 ± 0.002
Acid phosphatase	–	–	0.20 ± 0.02	0.31 ± 0.01	0.23 ± 0.01	–	–	–	–
α-Amylase	–	–	4.66 ± 0.28	8.94 ± 0.62	5.04 ± 0.41	–	–	–	0.669 ± 0.058
Amino acid arylamidase	0.36 ± 0.07	0.77 ± 0.20	0.30 ± 0.02	0.55 ± 0.06	0.32 ± 0.04	–	–	–	0.002 ± 0.0002
Aldolase	1.04 ± 0.13	3.32 ± 0.39	0.30 ± 0.03	0.64 ± 0.05	0.32 ± 0.03	0.008 ± 0.001	0.021 ± 0.002	0.008 ± 0.001	0.012 ± 0.002
Phosphohexose isomerase	6.12 ± 0.72	13.1 ± 2.5	3.37 ± 0.33	8.45 ± 1.1	4.23 ± 0.38	0.050 ± 0.004	0.13 ± 0.013	0.053 ± 0.005	0.068 ± 0.008
Protein	2.40 ± 0.27	5.24 ± 0.52	0.57 ± 0.04	1.13 ± 0.05	0.65 ± 0.04	0.042 ± 0.003	0.10 ± 0.006	0.043 ± 0.003	0.011 ± 0.001
Albumin	–	–	0.29 ± 0.02	0.56 ± 0.03	0.30 ± 0.02	0.024 ± 0.001	0.061 ± 0.005	0.022 ± 0.002	0.005 ± 0.0002

The same difference is obvious in rats when comparing the immobile state to passive motion; all enzymes and protein are transported at a higher rate (i. e. a threefold increase for malate dehydrogenase, creatine kinase, aldolase and phosphohexose isomerase). After passive motion, when the limbs are once more immobile, the values are not different from those in the immobile groups.

Liver lymph: catalytic enzyme activity, lymph/plasma ratio and enzyme transport

These results are summarized in table 3 a, 3 b and 3 c.

Generally only a few enzymes (alkaline phosphatase and amino acid arylamidase) have a lymph/plasma ratio of less than one. Protein ratios are approximately one.

Intestinal lymph: catalytic enzyme activity, lymph/plasma ratio and enzyme transport

These results are summarized in table 4 a, 4 b and 4 c.

Lymph/plasma ratios of less than one are observed for γ-glutamyl transferase, alanine aminotransferase, creatine kinase (in rat only) cholinesterase, alkaline phosphatase (except in rabbits), acid phosphatase (determined in rabbits only), amino acid arylamidase, protein and albumin.

Discussion

Lymph flow

A quantitative approach to lymph flow requires us to derive conclusions about the other lymph collecting vessels of the body and their drainage areas from our data on lymph flow of organ lymph (intestinal and hepatic) and of a lymph collecting vessel (thoracic duct). It is then possible to estimate relative proportional contributions to total body lymph flow.

A synopsis of published data on lymph flow is given in table 5. Four great lymph collecting systems drain the body's lymph and all eventually enter the intravascular space at the root of the neck. The thoracic duct drains lymph from the abdominal viscera and lower extremities (via the lumbar trunk), the left upper limb, the left side of the head and neck and parts of the heart. The right lymphatic duct drains most of the lungs, heart, pleural cavities, the right upper extremity and the right side of the head and neck (3, 18–21). Small proportions of the head,

Tab. 3. a) Catalytic enzyme activity concentrations (U/l), protein and albumin concentrations (g/l; $\bar{x} \pm$ SEM) in the liver lymph of the dog and the rabbit,
b) catalytic activity and mass concentration ratios ($\bar{x} \pm$ SEM) and
c) lymphatic transport of enzymes (U/h; $\bar{x} \pm$ SEM) and of protein and albumin (g/h; $\bar{x} \pm$ SEM) in the liver lymph.

	a) Concentration		b) Ratio		c) Transport	
	Dog	Rabbit	Dog	Rabbit	Dog	Rabbit
Lactate dehydrogenase	68.0 ± 6.6	226 ± 65	3.1 ± 0.38	4.5 ± 0.67	1.14 ± 0.18	0.24 ± 0.08
Malate dehydrogenase	124 ± 11	306 ± 26	2.6 ± 0.23	1.1 ± 0.07	2.08 ± 0.32	0.30 ± 0.02
Isocitrate dehydrogenase	6.0 ± 0.52	32.1 ± 2.1	1.7 ± 0.22	0.97 ± 0.09	0.12 ± 0.02	0.038 ± 0.004
Glutamate dehydrogenase	–	–	–	–	–	–
γ-Glutamyl transferase	2.3 ± 0.12	–	1.1 ± 0.07	–	–	–
Aspartate aminotransferase	22.4 ± 1.9	32.6 ± 3.7	1.8 ± 0.11	2.1 ± 0.27	0.48 ± 0.03	0.034 ± 0.004
Alanine aminotransferase	25.7 ± 1.7	42.8 ± 4.7	1.2 ± 0.09	1.2 ± 0.11	0.44 ± 0.03	0.046 ± 0.006
Creatine kinase	72.4 ± 9.4	331 ± 82	3.0 ± 0.30	3.2 ± 0.73	1.19 ± 0.18	0.33 ± 0.09
Adenylate kinase	53.1 ± 3.9	126 ± 20	2.4 ± 0.20	6.5 ± 1.11	0.84 ± 0.08	0.13 ± 0.02
Cholinesterase	2042 ± 136	169 ± 43	0.94 ± 0.02	2.0 ± 0.49	35.4 ± 3.6	0.17 ± 0.04
Alkaline phosphatase	61.4 ± 7.9	75.9 ± 4.8	0.91 ± 0.02	0.90 ± 0.09	0.79 ± 0.20	0.083 ± 0.005
Acid phosphatase	–	–	–	–	–	–
α-Amylase	1338 ± 53	2073 ± 597	0.79 ± 0.04	4.3 ± 1.9	20.1 ± 1.9	2.29 ± 0.63
Amino acid arylamidase	13.9 ± 0.56	23.5 ± 1.3	0.89 ± 0.05	0.86 ± 0.03	0.26 ± 0.03	0.026 ± 0.002
Aldolase	10.9 ± 0.55	–	1.4 ± 0.08	–	0.19 ± 0.03	–
Phosphohexose isomerase	125 ± 47.7	377 ± 44	2.3 ± 0.19	1.5 ± 0.20	2.23 ± 0.37	0.38 ± 0.05
Protein	63.2 ± 1.69	59.1 ± 1.2	0.96 ± 0.03	0.83 ± 0.03	0.95 ± 0.08	0.065 ± 0.006
Albumin	20.6 ± 0.58	32.2 ± 1.4	0.90 ± 0.04	0.88 ± 0.05	0.34 ± 0.03	0.036 ± 0.004

neck and forelegs are drained by the cervical and subclavian lymph ducts. The main contributors to thoracic duct lymph such as hepatic, intestinal lymph etc. are included in the table. Not included in this survey are values for heart and lung lymph, which drain into the thoracic and the right duct. Dog lung lymph, with a flow rate of 1.1–1.2 ml/h, is distributed nearly equally between these two ducts (3, 19, 20, 22, 23). Dog heart lymph accounts for 1.5–6 ml/h divided in unknown proportions between the two ducts (surveys: l. c. (2, 24)).

As can easily be seen from table 5, there is a wide range of lymph flow data for any given specific lymphatic. Differences in body weight probably do not explain this phenomenon, as there is no dependence on body weight for a given species over a wide weight range (3). The basis for these differences is probably two-fold: 1. presence or absence of anaesthesia and 2. variability in sampling techniques. Commonly used anaesthetics such as pentobarbital and halothane cause an over-all reduction in lymph flow which is reflected by a greater than 50% reduction in flow of thoracic duct lymph in anaesthetized dogs as compared to conscious dogs. This is due to inactivation of the "tissue pump", the main driving force of flow in the conscious state and by reduced capillary filtration (4, 25). The greatest anaesthesia-induced changes were seen in peripheral regions (i. e. skin, muscle). In anaesthetized dogs the only driving force for lymph flow is the "respiratory pump". Lymph from muscle is bypassed (26). The observed difference in thoracic duct flow between conscious and anaesthetized dogs therefore can be nearly totally accounted for by lymph from the hind legs. This was confirmed by the previous observation, that during passive motion of dog hind limbs, thoracic duct lymph increased to approximately the same degree as seen in the transition from the anaesthetized to the conscious state (4, 6). Further substantiation was done by direct determination of flow from the lumbar trunk which delivers leg lymph to thoracic duct (7). From the immobile leg, lymph flow is negligible (7, 27, 28). Our data on thoracic duct lymph in anaesthetized rats and rabbits with passive motion of hind legs vs. the immobile state, again confirm these previous findings.

The sparse data already available on thoracic duct flow in conscious dogs agree quite well with our data (4, 9, 26, 29). These data from conscious dog studies are not included in table 5. Data on lymph flow of anaesthetized dogs and rats (immobile) are numerous and our values approximately fit those reported (2, 3). The higher lymph flow we found in rabbits is almost certainly due to our technique, which, in contrast to others, leaves the mediastinum unimpaired.

Tab. 4a. Catalytic enzyme activity concentrations (U/l; $\bar{x} \pm$ SEM), protein and albumin concentration (g/l) in intestinal lymph of the dog, the rabbit and the rat.

	Dog	Rabbit	Rat
Lactate dehydrogenase	36.9 ± 3.3	170 ± 33	328 ± 42
Malate dehydrogenase	85.5 ± 7.9	290 ± 21	219 ± 20
Isocitrate dehydrogenase	4.1 ± 0.45	30.4 ± 3.4	–
Glutamate dehydrogenase	–	–	–
γ-Glutamyl transferase	1.8 ± 0.08	2.5 ± 0.28	–
Aspartate aminotransferase	18.7 ± 1.3	30.7 ± 3.8	49.3 ± 4.3
Alanine aminotransferase	25.0 ± 1.7	26.4 ± 2.1	12.1 ± 1.1
Creatine kinase	53.3 ± 3.7	258 ± 44	35.8 ± 4.3
Adenylate kinase	30.9 ± 1.2	130 ± 21	89.9 ± 5.7
Cholinesterase	1818 ± 133	72.5 ± 5.7	37.5 ± 3.5
Alkaline phosphatase	35.0 ± 1.6	144 ± 12	304 ± 22.7
Acid phosphatase	–	20.8 ± 2.0	–
α-Amylase	1242 ± 121	805 ± 84	–
Amino acid arylamidase	7.5 ± 0.39	28.1 ± 2.4	–
Aldolase	8.4 ± 0.26	16.9 ± 2.1	14.2 ± 1.5
Phosphohexose isomerase	78.4 ± 6.0	238 ± 29	251 ± 32
Protein	47.6 ± 1.7	55.5 ± 4.4	28.8 ± 1.4
Albumin	17.8 ± 0.61	28 ± 2.2	12.9 ± 0.55

Tab. 4b. Intestinal lymph/plasma catalytic activity and mass concentration ratios ($\bar{x} \pm$ SEM).

	Dog	Rabbit	Rat
Lactate dehydrogenase	1.4 ± 0.25	3.6 ± 0.68	4.8 ± 0.66
Malate dehydrogenase	1.7 ± 0.22	1.2 ± 0.12	6.1 ± 0.81
Isocitrate dehydrogenase	1.8 ± 0.20	0.90 ± 0.07	–
Glutamate dehydrogenase	–	–	–
γ-Glutamyl transferase	0.81 ± 0.05	0.88 ± 0.10	–
Aspartate aminotransferase	1.5 ± 0.15	1.6 ± 0.23	1.2 ± 0.10
Alanine aminotransferase	0.97 ± 0.07	0.88 ± 0.05	0.58 ± 0.06
Creatine kinase	2.9 ± 0.26	2.7 ± 0.45	0.45 ± 0.05
Adenylate kinase	1.7 ± 0.20	5.2 ± 0.69	1.9 ± 0.12
Cholinesterase	0.68 ± 0.04	0.76 ± 0.06	0.47 ± 0.05
Alkaline phosphatase	0.65 ± 0.03	1.5 ± 0.09	0.72 ± 0.05
Acid phosphatase	–	0.50 ± 0.04	–
α-Amylase	0.63 ± 0.05	2.6 ± 0.27	–
Amino acid arylamidase	0.67 ± 0.04	0.95 ± 0.06	–
Aldolase	1.1 ± 0.08	0.83 ± 0.08	1.1 ± 0.11
Phosphohexose isomerase	1.3 ± 0.17	0.96 ± 0.10	2.5 ± 0.29
Protein	0.78 ± 0.03	0.86 ± 0.06	0.54 ± 0.04
Albumin	0.76 ± 0.04	0.88 ± 0.07	0.67 ± 0.05

Tab. 4c. Lymphatic transport of enzymes (U/h; $\bar{x} \pm$ SEM), and of protein and albumin (g/h; $\bar{x} \pm$ SEM) in the intestinal lymph.

	Dog	Rabbit	Rat
Lactate dehydrogenase	2.28 ± 0.21	1.18 ± 0.28	0.23 ± 0.03
Malate dehydrogenase	5.91 ± 0.60	1.84 ± 0.26	0.17 ± 0.02
Isocitrate dehydrogenase	0.22 ± 0.03	0.21 ± 0.03	–
Glutamate dehydrogenase	–	–	–
γ-Glutamyl transferase	0.12 ± 0.01	0.014 ± 0.002	–
Aspartate aminotransferase	1.48 ± 0.18	0.20 ± 0.04	0.036 ± 0.004
Alanine aminotransferase	1.84 ± 0.18	0.17 ± 0.02	0.009 ± 0.001
Creatine kinase	4.00 ± 0.44	1.76 ± 0.42	0.022 ± 0.003
Adenylate kinase	2.33 ± 0.19	0.84 ± 0.18	0.061 ± 0.004
Cholinesterase	116 ± 9.0	0.37 ± 0.05	0.026 ± 0.003
Alkaline phosphatase	2.60 ± 0.23	0.97 ± 0.14	0.20 ± 0.02
Acid phosphatase	–	0.14 ± 0.02	–
α-Amylase	75.3 ± 4.9	4.96 ± 0.68	–
Amino acid arylamidase	0.50 ± 0.03	0.18 ± 0.02	–
Aldolase	0.61 ± 0.04	0.12 ± 0.02	0.010 ± 0.001
Phosphohexose isomerase	5.35 ± 0.50	1.58 ± 0.28	0.18 ± 0.022
Protein	3.55 ± 0.62	0.35 ± 0.06	0.020 ± 0.004
Albumin	1.33 ± 0.26	0.18 ± 0.04	0.009 ± 0.001

Tab. 5. Lymph flow (ml/h) in various lymph collecting vessels of man and several animals obtained from the literature. Data of the present study are not included.

	Man	Dog 20–35 kg body weight	Cat 3 kg body weight	Rabbit 2.5 kg body weight	Rat 200–350 g body weight	Mouse 18–31 g body weight
Cervical ducts	–	3.1– 6.0	–	1.5	0.08	–
Subclavian ducts	–	–	–	1.5	0.08	–
Right duct	1.5	1.2– 6.4	0.5	1.3	0.1	–
Thoracic duct	40–150	40 –65	7.3	4.0	0.45 –1.0	0.06–0.8
hepatic	–	15 –17	2.2	0.7–0.9	0.08 –0.21	–
intestinal	–	12 –13.6	4.6	–	0.3 –0.9	–
renal	–	2.2–14	–	–	0.024–0.06	–
lumbar trunk	–	36 –42	–	–	–	–

The following publications were reviewed:

Man: 37–43
Dog: 2, 3, 6, 7, 10, 21, 23, 44–46, 48–51
Cat: 34, 46
Rabbit: 13, 32, 52
Rat: 2, 3, 35, 36, 47, 53–58
Mouse: 59–64

The widest range of lymph flow has been reported for mice and is probably due to various pretreatments of the mice such as heparin injection and loading with saline and glucose solutions.

Especially for mice, where high flow rates in relation to body weight are seen, the applied long-term fistula technique used by other authors must have influenced lymph flow. This technique results in continuous fluid, protein, and cell loss and altered colloid osmotic forces. For our data, however, such effects are minimal, as we performed only short-term drainage in rabbits, rats and mice. These data, therefore, bear a greater resemblance to physiological conditions with the qualification that skeletal muscle interstitium is bypassed under anaesthesia and/or immobility.

Lymph draining parenchymal tissues in central regions (kidney, liver, intestine) was less affected by anaesthesia (30). The high intestinal lymph flow we saw in dogs can be accounted for by massage applied during sampling. Generally, liver and intestinal lymph flow data in the literature are rare or have not yet been reported (rabbit).

With respect to the contribution of thoracic duct lymph to total body lymph and the contributions of intestinal and hepatic lymph to thoracic duct lymph, certain misleading concepts and assumptions have been established over the years. "Thoracic duct lymph represents about 90% of total body lymph" is an often-used statement (3, 31). However, if it is true at all, it is true only for the anaesthetized state, where the total body lymph mainly consists of lymph from the abdominal and thoracal cavities. With anaesthesia *Hughes* et al. (32) found in rabbits the following proportions: thoracic duct 49%, right duct 15%, subclavian ducts 18%, cervical ducts 18%. In conscious animals, however, lymph flow from muscles increases in importance. This in fact increases thoracic duct lymph but also increases lymph flow in subclavian and cervical ducts, and therefore should not alter the proportions cited above for the anaesthetized rabbit. The 90% value therefore seems far too high. A value of 50–70% is probably more reliable. We are aware of the lack of direct experimental evidence for such a conclusion.

Another statement often made is that liver and intestinal lymph each contribute nearly equal parts to the total volume of lymph in the canine and feline thoracic duct (10, 33, 34). In rats, however, the hepatic contribution should amount to only 8.5–20% (35, 36). We suggest that in the anaesthetized state renal, cardiac and lung lymph contribute significantly to total thoracic duct lymph, whereas in the conscious state the lumbar trunk should be the major lymph contributor (see tab. 5).

In summary, thoracic duct lymph flow in anaesthetized animals is only half that in the conscious state due to bypassed muscular lymph. Passive motion of hind limbs in anaesthetized short-term fistulized animals results in thoracic duct flow similar to that seen in conscious animals. In relation to total body lymph flow, thoracic duct accounts for 50–70%. In the anaesthetized state, flow is mainly of visceral origin. In the conscious state or during passive motion, thoracic duct flow from muscle via the lumbar trunk is of major importance.

Lymph catalytic enzyme activity, lymph plasma/ratio and lymphatic enzyme transport

Thoracic duct

The relation of extracellular distribution and transport of enzymes to the measured intravascular catalytic activity is explained by the fact that enzymes released from cells in continuous physiological processes reach the intravascular space mainly via the lymphatics. Exceptions to this general rule are circulating blood cells, endothelial cells and, possibly, hepatocytes. For other tissues the possible direct interstitial to venous route of molecules of enzyme size is hindered by the heterogeneity of the capillary barrier that is characteristic of most organs. Generally stated, the back flux of protein at the microvascular level is negligible (65). Once a molecule has crossed the capillary wall it returns to the plasma via the lymph. Direct back-flow of enzyme molecules out of the interstitial space into the intravascular compartment via the capillaries is not compatible with a lymph/plasma ratio of 1. Albumin and other plasma proteins reach the interstitium by limited escape across capillary membranes. Their lymph/plasma ratio, therefore, must be less than 1. Ratios higher than 1 indicate that the substance measured derives primarily from the interstitial space. The molecular weight of the enzymes we studied is from 45 000 – 360 000 (except adenylate kinase). There is selective capillary membrane permeability for all molecules with molecular weights greater than 40 000 (66 – 68).

This suggests an interstitial origin, at least for those substances with a quotient which exceeds that of albumin.

Enzyme lymph/plasma ratios lower or similar to that of total protein (dogs) or albumin (other species) are observed under particular experimental conditions in certain species for alanine aminotransferase, cholinesterase, amino acid arylamidase, acid phosphatase and γ-glutamyl transferase. These enzymes, therefore, are probably partly of plasma origin. Relative organ contributions to thoracic duct lymph cannot be established from this data. All of the other enzymes we found in thoracic duct lymph are probably primarily released from tissue cells into the interstitial space and reach the intravascular space primarily via the lymphatic system.

In the section on lymph flow we have already discussed the negative effects of anaesthesia, immobility or long-term fistula technique on lymph flow. These factors are again reflected in marked differences of catalytic activity, lymph/plasma ratio and lymphatic enzyme transport between the anaesthetized vs. conscious vs. passive motion states. If animals are conscious or undergo passive motion of the legs (i. e. muscular lymph is present), the catalytic activities of enzymes such as lactate dehydrogenase, malate dehydrogenase, creatine kinase, aldolase and phosphohexose isomerase increase or at least remain constant.

Their lymph/plasma ratio also increases and their total enzyme transport increases 2 – 3-fold due to doubled lymph flow. These enzymes have been demonstrated to have high catalytic activity in striated muscle.

The catalytic activity of enzymes of mainly visceral origin (glutamate dehydrogenase, γ-glutamyl transferase, alanine aminotransferase, cholinesterase, amino acid arylamidase) and the concentration of protein and albumin decrease. Their lymph/plasma ratio decreases. Transport rate, however, occurs at a higher level as compared to anaesthetized or immobile states. This is due to accelerated lymph flow.

In the literature numerous reports on thoracic duct enzymatic composition, especially for dogs, are found (survey: l. c. (2)). Such studies were undertaken for a variety of reasons and have augmented the general validity of lymphatic transport of enzymes. In addition to the fact that in most cases only a few enzymes were measured, these studies were limited to anaesthetized dogs.

For anaesthetized rabbits, only some acid hydrolases are reported (52). For anaesthetized rats the only reported data are severely affected by methods of sample preparation (69). For mouse thoracic duct lymph, we recently reported selected catalytic activities in lymph and plasma of short-term fistulized animals (70).

The known data to date, at least for dogs, rabbits and rats, cannot be quantitatively considered.

The significance of transport of enzymes by the thoracic duct for the actual level of catalytic activity in plasma will be discussed later in relation to other contributing factors (e. g. release of enzymes from the ageing erythrocyte and the elimination constants of enzymes in plasma) (86, 87).

Liver lymph

A model for the direct delivery of newly synthesized liver proteins to the blood has been proposed. In this model, proteins are assumed to pass directly into the vascular compartment (sinusoidal space) without appreciable mixing with the extravascular pool

(Space of *Disse*). Hepatic lymph proteins therefore should originate mainly from the blood (71, 72). It remains, however, to explain why there are concentration differences between blood and lymph, with the lymph values always lower than plasma values. *Dive* et al. (73, 74) explain this phenomenon by two processes involved in the transfer of proteins from plasma to hepatic lymph. One is an indiscriminate bulk transport. The other is filtration through a semipermeable membrane with pores, in which the barrier for this double system of transfer probably resides in the capillary wall.

Our findings, however, that lymph catalytic activities for several enzymes are higher than those in plasma, is not compatible with the proposed general route of delivery of plasma proteins. In addition, we have not detected any relationship between the lymph/plasma ratio and the molecular weight of enzymes in lymph.

Four reasons may explain these findings.

1. False site of blood drawing, i. e. peripheral blood instead of hepatic vein blood.
2. Contamination of liver lymph with other organ lymph.
3. Enzymes in hepatic lymph originating from cells other than hepatocytes.
4. Different mechanisms for enzyme delivery and plasma protein delivery.

These four points are examined in detail below.

Ad 1

One could argue that enzymes and proteins in hepatic vein blood are found with higher catalytic activity and mass concentration than in peripheral blood. We would argue this contention with the following considerations. Assuming that all of aspartate aminotransferase found in plasma originates in the liver, an assumption which greatly overestimates the liver-derived aspartate aminotransferase by neglecting heart and skeletal muscle sources, the delivery should equal the elimination constant (k) for this enzyme in plasma. This constant equals 0.0035/min for dogs (75). If we accept that the liver is perfused with approximately 20% of the total plasma volume each minute (72), the rate of liver plasma perfusion is 270 ml/min in a 27 kg dog (50 ml plasma per kg body weight). The newly delivered aspartate aminotransferase is added at a rate of 0.0095 U/min ($k = 0.0035$/min; plasma catalytic concentration 10.1 U/l). The proportion of newly added enzyme to the steady state perfusion rate of 2.72 U/min equals 0.35%. This difference can hardly be detected analytically, considering an analytic variance of 6.8% for this enzyme (8). The difference between hepatic and peripheral venous blood, therefore, is of quite negligible significance.

Ad 2

Gastric, pancreatic and intestinal lymph can contaminate liver lymph. In gastric lymph *Keyl* et al. (76) found a lymph/plasma ratio for creatine kinase of 0.7. We found a ratio of about three in hepatic lymph. The high content of α-amylase in rabbit liver lymph would suggest a pancreatic contribution. There are, however, extrapancreatic sources of α-amylase, the salivary glands included, in man and animals. This can be concluded from liver and intestinal lymph vs. serum values of α-amylase as determined in pancreatectomized dogs (77). During sampling of hepatic lymph we never saw milky, opalescent contributions, which would point to intestinal origin.

It is suggested for these reasons, that we sampled essentially pure liver lymph.

Ad 3

In contrast to the plasma proteins found in hepatic lymph, enzymes should originate from nonparenchymal cells of the liver as well as hepatocytes. With respect to volume, surface area, and number, the nonparenchymal cells occupy about 10%, 27% and 50%, respectively, as compared to parenchymal cells (78, 79). Alkaline phosphatase, γ-glutamyl transferase, and lysosomal enzymes reveal three to nine-fold higher specific catalytic activities in nonparenchymal cells (80, 81); aldolase, alanine aminotransferase, aspartate aminotransferase, glutamate dehydrogenase and malate dehydrogenase are somewhat higher in parenchymal cells (79).

This endothelial lining cell net delimits the lumen of the sinusoids and the Space of Disse and should therefore release enzymes into both of these spaces.

Ad 4

It is difficult to envisage protein delivery from the liver cell to the plasma by a route which bypasses the Space of *Disse*, unless it be by immediate entry into the vascular compartment through the hepatic sinusoidal wall. The Space of *Disse*, limited on one side by the hepatocytes with numerous microvilli and on the other by endothelial cells, appears actually to be only part of an extensive labyrinth of intercellular

channels. These channels connect the more discrete Spaces of *Disse* and extend into the narrow spaces between the hepatocytes. Scanning images have shown that microvilli of hepatocytes project through the endothelial perforations or contact smaller fenestrations of the endothelial wall (82). Thus these ultrastructural findings are compatible with the proposed direct entry of proteins. Enzymes in liver lymph with a lymph/plasma ratio of greater than one, therefore must primarily derive from the Space of *Disse* and originate from either parenchymal and nonparenchymal cells. The site, however, where enzymes leave the hepatocyte, seems different from that of proteins. Some faces of the hepatocyte face the sinusoidal wall, while some front on the bile canaliculi and still others on the intercellular spaces. The latter may be the preferable site of enzyme release. It must, however, be assumed that enzymes from the Space of *Disse* can reach the sinusoidal space through the large number of wide fenestrations (up to 1.0 μm) and that they back-diffuse on a molecular weight basis mechanism as proposed for plasma proteins.

Dive et al. (73, 74) report on the strong relationship between the molecular weight of plasma proteins and their apparent lymph/plasma ratio, which for IgG_{2ab} (M_r = 160 000) and for α-macroglobulin (M_r = 820 000) in dogs have ratios of 0.65 and 0.506, respectively. Cholinesterase and α-amylase, with molecular weights of 360 000 and 45 000, respectively, define the upper and lower molecular weight limits of enzymes investigated so far (except adenylate kinase). If their occurrence in hepatic lymph is of plasma origin, they should reveal lymph/plasma ratios of about 0.55 and 0.70. These predicted quotients, however, were exceeded by all of these enzymes. We therefore conclude that not only enzymes with a lymph/plasma ratio above unity are of hepatic origin, but also those with a ratio above 0.70. This becomes even more plausible if one considers that adenylate kinase, with a molecular weight of 21 000, has a ratio of 2.4. This is not compatible with a plasma origin for this enzyme.

Such a theory of hepatocyte origin of enzymes helps to explain why we are unable to find any relation between the lymph/plasma ratio and the respective molecular weight. Secondly, the total amount of enzymes released by the hepatocytes is underestimated by measurement of the lymphatic enzyme transport alone, i. e. there is a small plasma transport component as well.

Despite this underestimation, the above mentioned data in the dog for the aspartate aminotransferase elimination constant (k), plasma volume, catalytic activity in plasma and lymphatic transport, showed that about 17% of the plasma-cleared aspartate aminotransferase is replaced by hepatic lymph transport. This is a surprisingly large amount, considering the higher catalytic concentration of this enzyme in heart, skeletal muscle and erythrocytes, all of which should contribute to the normal catalytic activity in plasma.

A similar calculation in the dog was done for a more "liver-specific" enzyme, alanine aminotransferase, with a clearance constant k = 0.0004/min (83) and a reference value in plasma of 18.3 U/l. This revealed that 74% of the plasma-cleared alanine aminotransferase is replaced by hepatic lymph transport. This suggests that enzyme transport from the liver to the circulating blood is mainly by lymphatic transport and not, as previously believed, by sinusoidal entry. *Woolley & Courtice* (71) have commented that "It is not possible to collect all the liver lymph in the rabbit". We would say that this applies to the dog as well. It is probable therefore that the reported values for aspartate and alanine aminotransferase are underestimates. For enzymes originating from the liver, both lymphatic and to a lesser degree direct sinusoidal pathways to the bloodstream are probably active under normal conditions.

Intestinal lymph

This lymph drains a well defined region. A specific origin for these enzymes in intestinal lymph should therefore be determinable. Comparison of lymph/plasma ratios for total protein and albumin with that of various enzymes clearly demonstrates that the enzymes are primarily released from tissue cells into the interstitial space. Their lymph/plasma ratio exceeds that of albumin, a completely plasma-derived protein. A unique exception to this rule is cholinesterase. This is rather logical, however, if one considers that this enzyme, a so-called plasma enzyme, primarly derives from the plasma. The findings on intestinal lymph for three different animal species generally confirm the data of *Friedel* et al. (47) for rat intestinal lymph. This group, however, found a relationship between the lymph/plasma ratio and the inverse of the molecular weight, whereas we did not in either species.

Regardless of the tissue in question, all plasma proteins equilibrate with the total extravascular compartment on a molecular weight basis, reflected by the respective lymph/plasma ratios, whose magnitude depends on the heterogeneity of the capillary barrier characteristics (3, 73, 74, 85). For enzymes in lymph, however, we have proven for thoracic duct, liver and

intestinal lymph that they are primarily released from tissue cells into the interstitial space. This process is independent of the molecular weight and is rather dependent on the relative abundance of an enzyme in the respective organ. The value of the lymph/plasma ratio for enzymes should, therefore, be a rough reflection of the enzyme pattern in the particular drained tissue (27). It was, therefore, not surprising that we found our highest ratios for creatine kinase, malate dehydrogenase and lactate dehydrogenase in the lumbar trunk, which drains the hind limbs (7). A molecular weight-dependent lymph/plasma ratio can only be expected if the respective proteins are of plasma origin. If they are primarily of interstitial origin and have reached the plasma via the lymphatics, as is the case for enzymes, such a relationship does not hold.

Acknowledgement

Supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 146, Versuchstierforschung.

References

1. Friedel, R., Diederichs, F. & Lindena, J. (1979) Release and extracellular turnover of cellular enzymes. In: Advances in clinical enzymology, (Schmidt, E., Schmidt, F. W., Trautschold, I. & Friedel, R., eds.) Karger, Basel pp. 70–105.
2. Lindena, J. & Trautschold, I. (1983) this J. *21*, 327–346.
3. Yoffey, J. M. & Courtice, F. C. (1970) Lymphatics, lymph and the lymphomyeloid complex. Academic Press, London.
4. Schad, H. & Brechtelsbauer (1977) Pflügers Arch. *371*, 25–31.
5. Lindena, J., Küpper, W. & Trautschold, I. (1984) Eur. J. Appl. Physiol. *52*, 188–195.
6. Lindena, J., Küpper, W., Friedel, R. & Trautschold, I. (1979) Enzyme *24*, 120–131.
7. Lindena, J., Küpper, W. & Trautschold, I. (1982) this J. *20*, 95–102.
8. Lindena, J. & Trautschold, I. † (1986) this J. *24*, 11–18.
9. Girardet, R. E. & Benninghoff, D. L. (1973) J. Surg. Res. *15*, 168–175.
10. Cain, J. C., Grindlay, J. H., Bollmann, J. L., Flock, E. V. & Mann, F. C. (1947) Surg. Gynecol. Obstet. *85*, 559–562.
11. Grindlay, J. H., Cain, J. C., Bollmann, J. L. & Mann, F. L. (1950) Surgery *27*, 152–158.
12. Zilversmit, D. B., Courtice, F. C. & Fraser, R. (1967) J. Ather. Res. *7*, 312–329.
13. Courtice, F. C. (1960) Austr. J. Exp. Biol. Med. Sci. *38*, 403–412.
14. Saldeen, T. & Linder, E. (1960) Acta Pathol. Microbiol. Scand. *49*, 433–437.
15. Warshaw, A. L. (1972) Gut *13*, 66–67.
16. Lee, J. S. (1981) Am. J. Physiol. *240*, G312–G316.
17. Lindena, J. & Trautschold, I. (1983) Lymphology. *16*, 243–246.
18. Leeds, S. E., Uhley, H. N., Basch, C. M., Rosenbaum, E. H. & Yoffey, J. M. (1971) Lymphology *4*, 53–57.
19. Meyer, E. C. & Ottaviano, R. (1974) Circ. Res. *35*, 197–203.
20. Vreim, C. E., Ohkuda, K. & Staub, N. C. (1977) J. Appl. Physiol. *43*, 894–898.
21. Glauser, F. L., Falls, R. K., Mathers, J. A. L. & Millen, J. E. (1981) Chest *79*, 217–221.
22. Parker, J. C., Falgout, H. J., Grimbert, F. A. & Taylor, A. E. (1980) Circ. Res. *47*, 866–875.
23. Vreim, C. E. & Ohkuda, K. (1977) J. Appl. Physiol. *43*, 899–901.
24. Michael, L. H. (1981) Life Sci. *29*, 1495–1505.
25. Schad, H., Flowaczny, H., Brechtelsbauer, H. & Birkenfeld, G. (1978) Pflügers Arch. *378*, 121–125.
26. Schad, H. & Brechtelsbauer, H. (1977) Pflügers Arch. *367*, 235–240.
27. Lindena, J., Küpper, W. & Trautschold, I. (1982) Enzyme *28*, 18–27.
28. Jacobsson, S. & Kjellmer, I. (1964) Acta Physiol. Scand. *60*, 278–285.
29. Doemling, D. B. & Steggerda, F. R. (1960) J. Appl. Physiol. *15*, 745–746.
30. Quin, J. W. & Shannon, A. D. (1975) Lymphology *8*, 126–135.
31. Ruszniak, J., Földi, M. & Szabo, G. (1969) Lymphologie. Physiologie and Pathologie der Lymphgefäße und des Lymphkreislaufes. G. Fischer, Stuttgart.
32. Hughes, R., May, A. J. & Widdicombe, J. G. (1956) J. Physiol. *132*, 384–390.
33. Nix, J. T., Mann, F. C., Bollmann, J. L., Grindlay, J. H. & Flock, E. V. (1951) Am. J. Physiol. *164*, 119–122.
34. Morris, B. (1956) Quart. J. Exp. Physiol. *41*, 318–325.
35. Mann, J. D. & Higgins, G. M. (1950) Blood *5*, 177–190.
36. Bollmann, J. L., Cain, J. C. & Grindlay, J. H. (1948) J. Lab. Clin. Med. *33*, 1349–1352.
37. Reynoso, G., Elias, E. G. & Mittelman, A. (1972) Am. J. Clin. Pathol. *56*, 707–712.
38. Werner, B. (1966) Acta Chir. Scand. *132*, 63–76.
39. Bierman, H. R., Byroni Jr., R. L., Kelly, K. H., Gilfillan, R. S., White, L. P., Freemann, N. E. & Petrakis, J. P. (1953) J. Clin. Invest. *32*, 637–649.
40. Girardet, R. E. & Benninghoff, D. L. (1977) Lymphology *10*, 36–44.
41. Lob, G., Liebich, H. G., Seifert, J., Rin, J., Coulin, C., Spelsberg, F., Walter, P. & Brendel, W. (1975) Anat. Anz. *137*, 120–133.
42. Evans, J. T. & Holyoke, E. D. (1975) Rev. Surg. *32*, 77–83.
43. Hallen, A. & Hansson, H. E. (1975) Scand. J. Thor. Cardiovasc. Surg. *9*, 56–61.
44. Islamov, I. I. (1970) Bull. Exp. Biol. Med. *70*, 1107–1108.
45. Uhley, H. N., Leeds, S. E., Sampson, J. J. & Friedman, M. (1963) Proc. Soc. Exp. Biol. Med. *112*, 684.
46. Courtice, F. C. (1956) Unpublished results. Citation in: Lymphatics, lymph and the lymphomyeloid complex. (Yoffey, J. M. & Courtice, F. C., eds.), Academic Press, London. p. 327.
47. Friedel, R., Bode, R., Trautschold, I. & Mattenheimer, H. (1976) this J. *14*, 119–128.
48. Heim, J. W. (1933) Am. J. Physiol. *103*, 553–558.
49. Polderman, H., McCarrell, J. D. & Becher, H. K. (1943) J. Pharm. Exp. Ther. *78*, 400–406.
50. Singh, H., Pepin, J., Appert, H. E., Pairent, F. W. & Howard, J. M. (1970) Arch. Surg. *100*, 253–256.
51. Bell, R. D. (1971) Lymphology *4*, 74–78.
52. Courtice, F. C., Adams, E. P., Shannon, A. D. & Bishop, D. M. (1974) Quart. J. Exp. Physiol. *59*, 31–42.
53. Schooley, J. (1958) Proc. Soc. Exp. Biol. Med. *99*, 511–513.
54. Reinhardt, W. O. (1945) Proc. Soc. Exp. Biol. Med. *58*, 123–124.
55. Reinhardt, W. O. (1946) Anat. Rec. *94*, 197–211.

56. Gartemann, H., Dennhardt, R. & Stöckert, H.-G. (1979) Role of the intestinal lymphatic system in the absorption of water, electrolytes and hexoses. In: Lymphology (Malek, P., Bartos, V., Weissleder, H. & Witte, M. H., eds.). Georg Thieme Publishers, Stuttgart pp. 85–88.
57. Friedman, M., Byers, S. O. & Omoto, C. (1956) Am. J. Physiol. *184*, 11–17.
58. Hargens, A. R., Tucker, B. J. & Blantz, R. C. (1977) Am. J. Physiol. *233*, F269–F273.
59. Shrewsbury, M. M. (1959) Proc. Soc. Exp. Biol. Med. *101*, 492–494.
60. Gesner, B. M. & Gowans, J. L. (1962) Brit. J. Exp. Pathol. *43*, 424–430.
61. Boak, J. L. & Woodruff, M. F. A. (1965) Nature *205*, 396–397.
62. Morse, S. J. & Riester, S. K. (1967) J. Exp. Med. *125*, 619–631.
63. Deaton, J. G. (1972) Lymphology *5*, 115–120.
64. Mandel, M. A. (1967) Proc. Soc. Exp. Biol. Med. *126*, 521–524.
65. Lassen, N. A., Parving, H.-H. & Rossing, N. (1974) Microvasc. Res. *7*, i–iv.
66. Grotte, G. (1956) Acta Chir. Scand. *211* (Suppl.) 1–84.
67. Pappenheimer, J. R. (1953) Physiol. Rev. *33*, 387–423.
68. Rutili, G. & Arfors, K.-E. (1977) Acta Physiol. Scand. *99*, 1–8.
69. Vogel, G. & Stöckert, J. (1963) Pflügers Arch. Ges. Physiol. *277*, 236–241.
70. Lindena, J. & Trautschold, I. (1983) Lymphology *16*, 247–249.
71. Wolley, G. & Courtice, F. C. (1962) Austr. J. Exp. Biol. *40*, 121–128.
72. Smallwood, R. A., Jones, E. A., Craigie, A., Raia, S. & Rosenoer, V. M. (1968) Clin. Sci. *35*, 35–43.
73. Dive, Ch. C., Nadalini, A. C. & Heremans, J. F. (1971) Lymphology *4*, 133–139.
74. Dive, C., Nadilini, R. A., Vaerman, J. P. & Heremans, J. F. (1974) Eur. J. Clin. Invest. *4*, 241–246.
75. Visser, M. P., Krill, M. T., Willems, G. M. & Hermens, W. T. (1981) Cardiovasc. Res. *15*, 35–42.
76. Keyl, M. J., Chang, A. C. K. & Dowell, R. T. (1981) Lymphology *14*, 118–121.
77. Singh, H., Pepin, J., Appert, H. E., Pairent, F. W. & Howard, J. M. (1969) Arch. Surg. *99*, 80–82.
78. Reith, A. (1979) Enzyme topology and morphometry. In: Advances in clinical enzymology (Schmidt, E., Schmidt, F. W., Trautschold, I. & Friedel, R., eds.) Karger, Basel pp. 63–69.
79. Schmidt, E., Schmidt, F. W., Ohlendorf, S. & Raupach, R. (1983) Effect of diet and cell damage on enzyme activities in different hepatic cells. In: Progress in Clinical Enzymology, Vol. II (Goldberg, D. M. & Werner, M., eds.). Masson, New York pp. 53–61.
80. Munthe-Kaas, A. C., Berg, T., Seglen, P. O. & Seljelid, R. (1975) J. Exp. Med. *141*, 1–10.
81. Wilson, P. D., Watson, R. & Knook, D. L. (1982) Gerontology *28*, 32–43.
82. Motta, P. & Porter, K. R. (1974) Cell Tiss. Res. *148*, 111–125.
83. Visser, M. P., Krill, M. T. A., Willems, G. M. & Hermens, W. Th. (1982) Lab. Anim. *16*, 248–255.
84. Nordbeck, H., Kahles, H., Preusse, C. J. & Spiekermann, P. G. (1977) J. Mol. Med. *2*, 255–263.
85. Olszewski, W. L. & Engeset, A. (1978) Lymphology *11*, 156–164.
86. Lindena, J., Wittenberg, H., Diederichs, F. & Trautschold, I. † (1986) this J. *24*, 49–59.
87. Lindena, J., Diederichs, F., Wittenberg, H. & Trautschold, I. † (1986) this J. *24*, 61–71.

Joachim Lindena, DVM
Abteilung Klinische Biochemie
Medizinische Hochschule Hannover
Konstanty-Gutschow-Straße 8
D-3000 Hannover 61